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Characterization of Monoclonal Antibodies Against Hepatitis C Virus Nonstructural Protein 3: Different Antigenic Determinants From Human B Cells

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The nonstructural (NS3) region protein of hepatitis C virus (HCV) possesses major B-cell epitopes that induce antibodies after infection. To elucidate further the characteristics of these B cells and their role in the immune regulation of HCV infection, T9 (portion of NS3 region, amino acids [a.a.] 1188–1493)-specific monoclonal antibodies were derived and mapped for B-cell antigenic determinants with recombinant proteins. A total of 10 T9-specific hybridomas were generated and tested for B-cell antigenic determinants. To analyze the B-cell antigenic determinants, eight recombinant proteins including NS3-e (a.a. 1175–1334), NS3-a′ (a.a. 1175–1250), NS3-a (a.a. 1251–1334), NS3-b (a.a. 1323–1412), NS3-c (a.a. 1407–1499), NS3-a/b (a.a. 1251–1412), NS3-bc (a.a. 1323–1499), and NS3-abc (a.a. 1251–1499) encoded by NS3-region internal clones were expressed and tested for immunoblotting. The data suggested IgG hybridomas recognized NS3-a, NS3-a′, or NS3-b protein by immunoblotting. By contrast, the NS3-e protein bears the major antigenic determinant recognized by human sera. Half of the hybridomas were found to react with protein NS3-a′, which is not a major B-cell antigenic determinant in humans. These data suggested that conformational epitopes in vivo may be important for B-cell recognition. ***J. Med. Virol. 57:345–350, 1999.*** © 1999 Wiley-Liss, Inc.

KEY WORDS: hepatitis C virus; monoclonal antibodies

INTRODUCTION

After hepatitis C virus (HCV) genome was identified, many studies on the definition of HCV sequences and antibodies production have been documented [Choo et al., 1989]. Using commercially available enzyme-linked immunosorbent assay (ELISA) kits, HCV has been found to be responsible for about 60–90% non-A, non-B post-transfusion hepatitis [Alter et al., 1989; Chen et al., 1990]. Unlike hepatitis A and B viral infection, hepatitis C infection has been characterized by an unusually high frequency of persistent infection after the acute stage, and about 20% of the patients may eventually develop cirrhosis and hepatoma. Because of the low viral load in the sera of HCV patients, reverse transcription-polymerase chain reaction (RT-PCR) is needed to detect the virus in vivo [Okamoto et al., 1992a]. The Taiwanese HCV genome was cloned and most of the viral proteins were expressed [Chen et al., 1992].

Several viral antigens such as C100-3 (NS4 region), C22 (the N-terminus of the core antigen), e1 (part of the E1 region), e2 (part of the E2 region), C33C (most of the NS3 region), and ns5 (part of the NS5 region) proteins have been used in commercial kits to detect anti-HCV antibodies in patients [Katayama et al., 1990; Chien et al., 1992; Nakatsuji et al., 1992]. A high prevalence of antibodies against C22, C33C, and ns5 polypeptides was noted in HCV [Aach et al., 1991; Nakatsuji et al., 1992]. It has thus been well documented that NS3 region protein has the major B-cell epitope in HCV infection. We suggested previously that one of the proteins (NS3-e, a.a. 1175–1334) is the major antigenic determinant recognized by human B cells, and antibody to one of the proteins (protein NS3-a, a.a. 1250–1334) was less common in cirrhotic patients than that in chronic hepatitis patients [Hwang et al., 1996]. Up to now, only very few monoclonal antibodies against the NS3 region protein have been reported.

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Hybridomas specific for NS3 protein were developed and B-cell antigenic determinants recognized by monoclonal antibodies were characterized. These monoclonal antibodies may be applied for immunohistochemical study and determination of HCV antigen in the future.

MATERIALS AND METHODS

Immunization of Mice

To develop hybridomas specific for T9 antigen, Balb/c mice were immunized subcutaneously with 25 μg Ag in 100 μl phosphate-buffered saline (PBS) with complete Freund's adjuvants as the primary immunization. Ten days after the primary immunization, the mice were boosted by intraperitoneal injection with 50 μg Ag in 100 μl PBS mixed with incomplete Freund's adjuvant. Then 25 μg Ag in 100 μl PBS was injected with incomplete Freund's adjuvant by subcutaneous and intraperitoneal injection alternately. Seven days after each injection, the sera of mice were assayed for the titer of specific antibodies by ELISA. Intravenous injection with 25 μg Ag in 100 μl PBS only was used as the final boost immunization 3 days before a hybridoma fusion.

ELISA for Anti-T9 Antibody

Microtiter plates were coated with 0.5 μg T9 antigen in 100 μl carbonate coating buffer per well and incubated at 4°C overnight. The microtiter plates were washed twice with 1× PBS containing 0.05% Tween 20. Then the plates were blocked with blocking solution (1% bovine serum albumin [BSA]–0.05% Tween 20 in 1× PBS) and washed three times after incubation. One hundred microliters of sample was added to each well and washed six times after reaction. Then 100 μl anti-mouse IgG or IgM antibody was added and then washed six times. Finally, 100 μl of ABTS (2.2′-azino-bis-3-ethylbenzthiazoline-6-sulfonic acid) substrate solution was added and incubated at room temperature (RT) for about 30 min; the reaction was then stopped with 100 μl of 5% sodium dodecyl sulfate (SDS). Plates were read with a microplate reader (Metertech Inc., Taipei, Taiwan).

Purification of NS-3 Region Gene-Coded Proteins

To purify the proteins used in T-cell cloning and epitope mapping, plasmids containing pET-HCV sequences were transformed into *Escherichia coli* BL21(DE3). Cells were grown overnight at 37°C in Luria broth (L broth) containing ampicillin (50 μg/ml). On the second day, the cultures were diluted 1:100 with L broth containing 300 μg/ml ampicillin, six times the concentration of that normally used to increase the efficiency of induction. The cultures were grown to an OD600 = 0.5–1 unit. After addition of 1 mM isopropyl-β-D-thiogalactoside (IPTG), cells were incubated at 37°C for an additional 3 hr and then harvested by centrifugation. Cells were broken by a French Press at 1,000 psi, inclusion bodies were obtained by centrifugation at 7,000 rpm for 15 min, further dissolved in SDS-sample buffer and analyzed by SDS-polyacrylamide gel electrophoresis (PAGE).

Establishment of T9-Specific Monoclonal Antibodies

Single cell suspensions of spleen cells were prepared and fused with F-0 myeloma cells in the presence of polyethylene glycol using standard protocols. A ratio of 3:1 spleen cells to myeloma cells was used and cells plated at 2×10^5 per well in 96-well plates in RPMI 1640 medium containing HAT supplement (Sigma, St. Louis, MO). Hybridomas were cloned in soft agar or by limiting dilution (0.5–1 cell/well). Isotype analysis was carried out with radial immunodiffusion (Serotec Ltd., Oxford, England).

Immunoblot Analysis

Protein of the inclusion bodies was resolved by 12.5% SDS-PAGE and electrotransferred to a nitrocellulose filter. The filter was blocked with 5% nonfat milk dissolved in TBS buffer (0.05M Tris-HCl, 0.9% NaCl, pH 7.6) at RT for 2 hr, followed by incubation with supernatants of hybridomas or patients' sera (1:500 dilution) at RT for 2 hr. After six extensive washes with TBST (0.25% Tween-20 in TBS), goat anti-human IgG conjugated with horseradish peroxidase was added at a 1:10,000 dilution and incubated at RT for 2 more hr. The filter was then washed six times with TBST, and developed with the electrochemiluminescence (ECL) detection reagent (Amersham, Buckinghamshire, UK).

Immunoprecipitation of NS-3 Region Proteins With Monoclonal Antibodies

To analyze further the ability of monoclonal antibodies to immunoprecipitate NS3 region protein, in vitro transcription and translation was carried out as described previously [Chiang et al., 1998]. Radiolabelled (^{35}S-) protein in the supernatant was incubated with ascites of hybridomas in buffer containing 150 mM NaCl, 50 mM Tris pH 7.5, 5 mM ethylenediamine tetraacetic acid (EDTA), 1% NP-40, 0.5% deoxycholate, 0.1% SDS, 1 mM PMSF, 50 μg/ml aprotinin, and 50 μM leupeptin at 4°C for 1 hr on an orbital shaker. Then, 20 μl of protein A-Sepharose beads were added to the tube and incubated for 1 hr. The mixture was centrifuged at 7,500 rpm for 30 sec and the supernatant was discarded. After four washes, the pellet was dissolved in 20 μl SDS/sample buffer and incubated for 5 min at 100°C. The sample was loaded onto a SDS-PAGE gel and analyzed. The gel was immersed in fix buffer and autoradiographed.

Immunofluorescence Staining Assay

The NS3 protein expressing Huh 7 (NS3/Huh7) cells were plated on the slides and then washed twice with PBS, pH 7.2. A total of 15 μl of individual monoclonal antibodies were added to each slide and incubated in humidified chambers at 37°C for 30 min. The slides were then washed three times with PBS and fluorescein isothiocyanate (FITC)-conjugated secondary anti-

TABLE I. Recombinant Antigens Recognized by T9 Protein-Specific Hybridomas

Hybridomas	Isotype	Recombinant proteins									Antigenic determinant
		a	a′	b	c	e	ab	bc	abc	T9	
3E10.3	γ1	+	−	−	−	+	+	−	+	+	a
3G10.9	γ1	−	+	−	−	+	−	−	−	+	a′
3H12.3	γ1	−	+	−	−	+	−	−	−	+	a′
4B9.1	γ1	−	+	−	−	+	−	−	−	+	a′
6E4.5	γ1	+	−	−	−	+	+	−	+	+	a
3G3.1	γ1	−	+	−	−	+	−	−	−	+	a′
2D12.4	γ1	+	−	−	−	+	+	−	+	+	a
10E11.2	γ1	−	−	+	−	±	+	+	+	+	b
9A1.4	γ1	−	+	−	−	+	−	−	−	+	a′
3G5.6	μ	−	−	+	−	+	+	−	+	+	b

body was added and incubated in humidified chambers at 37°C for another 30 min. After three washes with PBS, the slides were counterstained with 0.01% of Evan's blue, mounted with 90% glycerol in PBS, and observed with a fluorescence microscope.

RESULTS

Establishment of T9-Specific Monoclonal Antibodies

Ten T9-specific hybridomas were derived and studied for their antigen specificity by both ELISA and immunoblotting. The result of the T9-specific hybridomas is summarized in Table I. All the IgG anti-T9 hybridomas were found with IgG1 subclass by radial immunodiffusion. In addition, hybridoma cells were injected into the peritoneal cavity of Balb/c mice with the cell number of 1×10^6 cells per mouse. The ascitic fluid was collected 2 weeks later and used in immunoprecipitation and immunofluorescence staining.

B-Cell Antigenic Determinant Analysis of T9-Specific Monoclonal Antibodies

To further determine antigenic regions recognized by T9-specific monoclonal antibodies, different expressed NS3 region proteins were used for immunoblotting. In summary, three different antigenic regions, protein NS3-a′, NS3-a, and NS3-b, were noted among the monoclonal antibodies (Fig. 1 and Table I). All the hybridomas reacted with protein NS3-e, an observation similar to that of human study (the positive rate of anti-NS3-e was about 93% in HCV patients, Hwang et al., 1996). Five of 10 hybridomas reacted with protein NS3-a′ (a.a. 1175–1250). In humans, no antibody response against the region a.a. 1007–1250, which covered NS3-a′ region (1175–1250), was noted among 90 HCV patients screened. In contrast, clones 3G5.6 and 10E11.2 recognized both NS3-e and NS3-b, which overlapped by 12 amino acids (a.a. 1323–1334, Fig. 2). This finding suggested a.a. 1323–1334 may represent a B-cell antigenic determinant recognized by the monoclonal antibodies. In addition, one major epitope around a.a. 1250–1251 was not observed in our monoclonal antibodies. Certain human sera (15% of HCV patients) reacted only with protein NS3-e but not NS3-a or NS3-a′, suggesting a sequence at a.a. 1250–1251 is probably one of the B-cell antigenic determinants (Fig. 3).

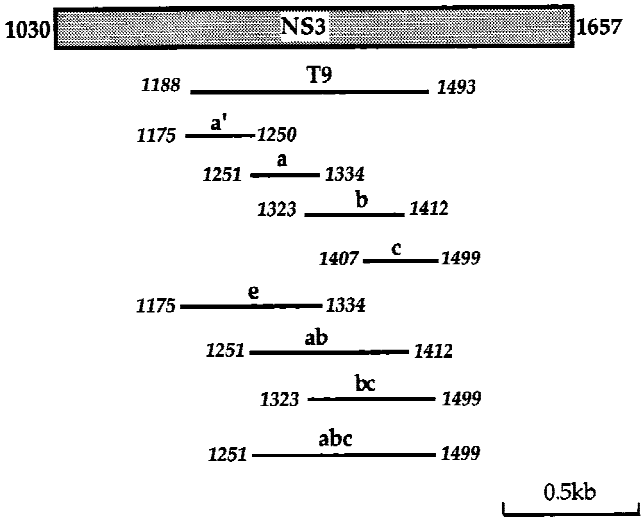

Fig. 1. Location of total eight recombinant antigens in the nonstructural region protein of hepatitis C virus (HCV-NS3). Amino acid sequences are shown in *italic*.

Immunoprecipitation Analysis

The results suggested that all the clones, 9A1 (anti-NS3-a′), 6E4.5 (anti-NS3-a), 10E11.2 (anti-NS3-b), and 3G10.9 (anti-NS3-a′), can immunoprecipitate the NS3 region proteins (Fig. 4). However, clone 3G10.9 showed the highest ability to react and immunoprecipitate the NS3 region proteins. Monoclonal antibodies with different affinities may be used for two-site ELISA or affinity column to assay the HCV antigens.

Immunofluorescence Staining

The data of immunofluorescence staining are summarized in Figure 5. The results showed that clone 3G10.9, with the highest reactivity in the immunoprecipitation study, also demonstrated the strongest response in immunofluorescence staining of NS3 region protein-expressed cells.

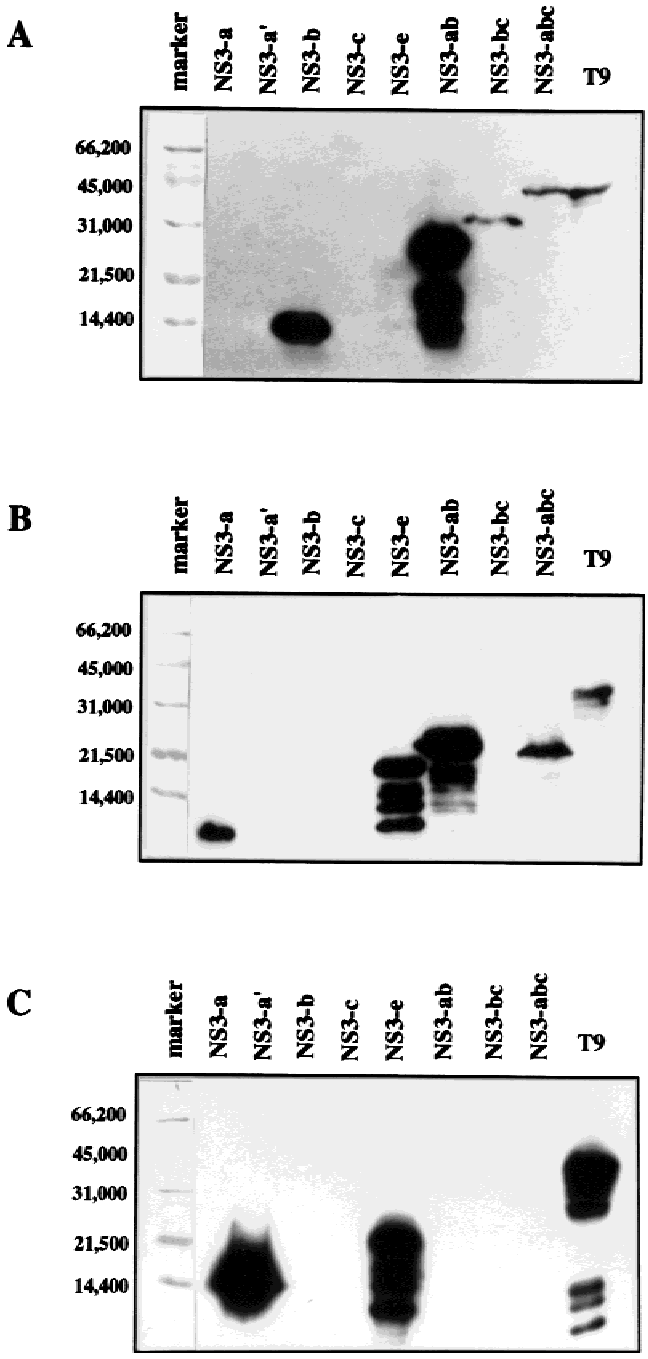

Fig. 2. The pattern of antigenic determinants determined by immunoblot. **A:** Clone 10E11.2 reacted with proteins NS3-b; **B:** clone 6E4.5 recognized antigenic region of protein NS3-a; **C:** clone 3G10.9 reacted with protein NS3-a′.

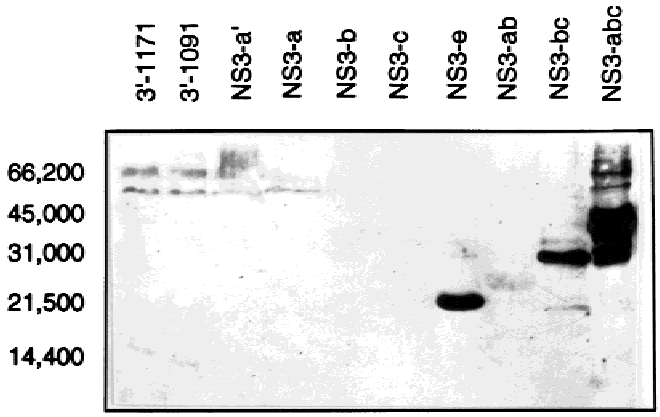

Fig. 3. Human sera reacting with nonstructural (NS3) region protein. The data showed that the sera reacted only with the protein NS3-e, but not NS3-a′ or NS3-a, suggesting that one B-cell antigenic determinant resides at about amino acid 1250–1251.

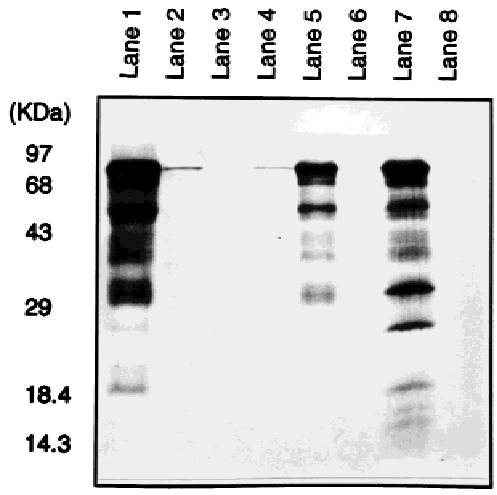

Fig. 4. Immunoprecipitation of nonstructural (NS3) region proteins by the monoclonal antibodies. **Lane 1:** mouse polyclonal anti-serum; **lane 2:** clone 9A1.4; **lane 3:** clone 6E4.5; **lane4:** 10E11.2; **lane 5:** 3G10.9; **lane 6:** anti-HDV antisera; **lane 7:** anti-HCV antisera; **lane 8:** negative control. Clone 3G10.9 (anti-NS3-a′) was found to have the strongest response in immunoprecipitation assay.

DISCUSSION

HCV has low viral load in the peripheral blood, which can be detected only by sensitive PCR [Okamoto et al., 1992b; Nakatsuji et al., 1992]. In addition, hypermutation has been reported to occur in HCV infection, which might cause a lack of neutralizing antibody after infection [Okamoto et al., 1992]. Several mechanisms such as escape mutation of CTL epitopes, lack of effective neutralizing antibody, and dysregulation of T helper cells have been suggested to play important roles in the chronicity of HCV infection [Farci et al., 1992; Weiner et al., 1992]. Further dissection of cellular and humoral immune responses of HCV infection might shed light on the understanding of the pathogenesis of this infection. Accordingly, we generated and characterized a panel of monoclonal antibodies against HCV NS3 region protein (a.a. 1175–1334).

It has been well documented that the protein encoded by NS3 region genome possessed the major B-cell epitopes during HCV infection [Mosely et al., 1990]. The NS3 region encoded-protein was found to possess the activity of serine protease, which is important for assembly of HCV polyprotein [Bartenschlager et al., 1993; Tomai et al., 1993]. Data also showed that multiple T- and B-cell antigenic determinants were found

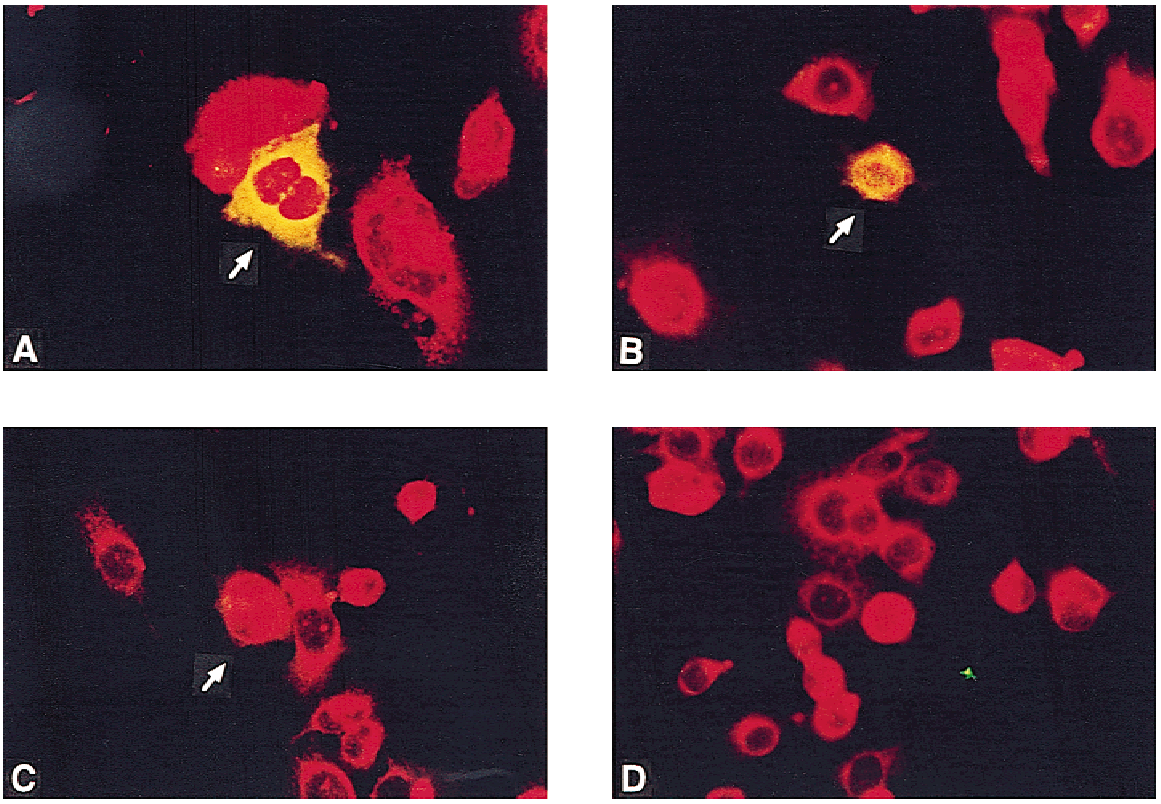

Fig. 5. Immunofluorescence staining of NS3/Huh 7 cells with monoclonal antibodies. The results suggested that different monoclonal antibodies, 3G10.9 **(A)**, 6E4.5 **(B)**, 10E11.2 **(C)**, and negative control **(D),** had various responses to immunofluorescence staining. Clone 3G10.9 was found to have the strongest response in immunofluorescence staining of NS3 region protein-expressing cells.

inside the NS3 region [Yang et al., 1995; Hwang et al., 1996]. Peripheral blood mononuclear cells of about 66% of HCV patients proliferated to T9 antigen and multiple T-cell antigenic determinants in the NS3 region [Yang et al., 1995; Chiang et al., 1998]. The hybridomas in our study were all found to be IgG_1, which has been suggested to be induced by TH2-related cytokines in mice. In contrast, human peripheral blood and intrahepatic HCV-specific cytotoxic T cells are primarily of type 1 cytokine profile [Napoli et al., 1996; Chisari, 1997]. However, one report also demonstrated that NS3 protein immunization in mice resulted in predominant IgG1 antibody and paradoxical TH0 and TH1 cytokines [Sallberg et al., 1996]. More studies are needed to clarify further the interaction between HCV protein-reactive B cells and T cells.

Several studies concerning B-cell epitope analysis of HCV core antigens have been documented; however, only few studies concerning B-cell antigenic determinants in NS-3 region have been reported [Nasoff et al., 1991; Koskinas et al., 1994; Siemoneit et al., 1994; Moradpour et al., 1996]. A previous study has suggested multiple B-cell antigenic regions (NS3-e, NS3-a, NS3-a/b, NS3-b/c) identified by human B cells [Hwang et al., 1996]. The B-cell antigenic determinants (a.a. 1359–1449) and (a.a. 1363–1454) in the NS3 region have also been reported [Mondelli et al., 1994; Claeys et al., 1995]. These two proteins overlap with the NS3-b and NS3-c regions in the present study; however, the B-cell antigenic determinant of the hybridomas was more limited to around a.a. 1323–1334. One of the major B-cell antigenic determinants was protein NS3-e (a.a. 1175–1334), which can be recognized by both human sera (93%) and murine monoclonal antibodies (100%). However, half of the hybridomas reacted with protein NS3-a′, which was not a dominant B-cell antigenic region recognized by human sera. There has been some concern about the conformation of HCV antigen used in immunization of mice, because most of the recombinant antigens were processed and denatured. It has been suggested that both HCV-infected humans and murine anti-NS3 antibodies were directed predominantly to conformational determinants [Sallberg et al., 1996]. Although NS3-specific antibody is not the major neutralizing antibody during HCV infection, the data in the present study raise an issue that vaccination with denatured recombinant antigens might not generate protective antibody against viral infection. In the future, investigating the conformation of antigen and related immune response may be critical for determining effectiveness of vaccine.

The mechanisms of HCV viral replication and protein expression in vivo are yet to be defined. Histochemical study has suggested that only few virus-infected hepatocytes can be detected in the livers of patients with HCV compared to those of patients with

HBV [Hiramatsu et al., 1992]. There are only a few monoclonal antibodies available for immunohistochemical staining to detect viral protein on frozen tissue [Hiramatsu et al., 1992; Santolini et al., 1994; Moradpour et al., 1996]. One of our monoclonal antibody has shown strong reactivity against hepatocytes transfected with NS3 region (Fig. 5A), and thus may be useful in further studies.

The monoclonal antibodies described above might be applied for the determination of antigen level, purification of antigen, and immunohistochemical examination of infected human liver tissue. Furthermore, the data also suggested that hybridomas derived in mice recognized different B-cell epitopes from HCV patients' sera and conformational epitopes in vivo may be important for B-cell recognition.

REFERENCES

Aach RD, Stevens CE, Hollinger FB, Mosely JW, Peterson DA, Taylor PE, Johnson RG, Barbosa LH, Nemo GJ. 1991. Hepatitis C virus infection in post-transfusion hepatitis: an analysis with first- and second-generation assay. N Engl J Med 325:1325–1329.

Alter HJ, Purcell RH, Shih JW, Melpolder JC, Houghton M, Choo Q-L, Kuo G. 1989. Detection of antibody to hepatitis C virus in prospectively followed transfusion recipients with acute and chronic non-A, non-B hepatitis. N Engl J Med 321:1494–1500.

Bartenschlager R, Ahlborn-Laake L, Mous J, Jacobsen H. 1993. Nonstructural protein 3 of the hepatitis C virus encodes a serine-type proteinase required for cleavage at the NS3/4 and NS4/5 junctions. J Virol 67:3835–3844.

Chen D-S, Kuo G, Sung J-L, Lai M-Y, Sheu J-C, Chen P-J, Yang P-M, Hsu H-M, Chang M-H, Chen C-J, Hahn L-C, Choo Q-L, Wang T-H, Houghton M. 1990. Hepatitis C virus infection in the area hyperendemic for hepatitis B and chronic liver disease: the Taiwan experience. J Infect Dis 162:817–822.

Chen P-J, Lin M-H, Tai K-F, Liu P-C, Lin C-J, Chen D-S. 1992. The Taiwanese hepatitis C virus genome: sequence determination and mapping the 5′ termini of viral genomic and antigenic RNA. Virology 188:102–113.

Chiang B-L, Yang P-M, Hwang L-H, Wang J-M, Kao S-F, Pan C-H, Chi W-K, Chen P-J, Chen D-S. 1998. Establishment and characterization of NS3-specific T cell clones from a patient with chronic hepatitis C. J Biomed Sci 5:290–296.

Chien DY, Choo Q-L, Tabrizi A, Kuo G, McFarland J, Berger K, Lee C, Shuster JR, Nguyen T, Moyer DL, Tong M, Furuta S, Omata M, Tegtmeimer G, Alter H, Schiff E, Jeffers LM, Houghton M, Kuo G. 1992. Diagnosis of hepatitis C virus (HCV) infection using an immunodominant chimeric polypeptide to capture circulating antibodies: reevaluation of the role of HCV in liver disease. Proc Natl Acad Sci USA 89:10011–10015.

Chisari FV. 1997. Cytotoxic T cells and viral hepatitis. J Clin Invest 99:1472–1477.

Choo Q-L, Kuo G, Weiner AJ, Overby LR, Bradley DW, Houghton M. 1989. Isolation of a cDNA derived from a blood-borne non-A, non-B viral hepatitis genome. Science 244:359–362.

Claeys H, Volckaerts A, Mertens W, Liang Z, Fiten P, Opdenakker G. 1995. Localization and reactivity of an immunodominant domain in the NS3 region of hepatitis C virus. J Med Virol 45:273–281.

Farci P, Alter HJ, Govindarajan S, Wong DC, Eagle R, Lesniewski RR. Mushahwar IK, Desai SM, Miller RH, Ogata N, Purcell RH. 1992. Lack of protective immunity against reinfection with hepatitis C virus. Science 258:135–140.

Hiramatsu N, Hayashi N, Haruna Y, Kasahara A, Fusamoto H, Mori C, Fuke I, Okayama H, Kamada T. 1992. Immunohistochemical detection of hepatitis C virus-infected hepatocytes in chronic disease with monoclonal antibodies to core, envelop and NS3 regions of the hepatitis C virus genome. Hepatology 16:306–311.

Hwang L-H, Yang P-M, Lai M-Y, Chiang B-L, Kao J-H, Wang J-T, Lee S-Y, Chian H-M, Chi W-K, Chu Y-D, Chen P-J, Chen D-S. 1996. Identification of dominant humoral antigenic determinants within the hepatitis C virus nonstructural protein 3. J Infect Dis 174: 173–176.

Katayama T, Kikuchi S, Tanaka Y, Saito I, Miyamura T, Choo Q-L, Houghton M, Kuo G. 1990. Blood screening for non-A, non-B hepatitis C virus antibody assay. Transfusion 30:374–376.

Koskinas J, McFarlane BM, Nouri-Aria KT, Tibbs CJ, Mizokami M, Donaldson PT, McFarlane IG, Williams R. 1994. Cellular and humoral immune reactions against autoantigens and hepatitis C viral antigens in chronic hepatitis C. Gastroenterology 107:1436–1442.

Mondelli MU, Cerino A, Boender P, Oudshoorn P, Middeldrop J, Fipaldini C, La Monica N, Habets W. 1994. Significance of the immune response to a major conformational B-cell epitope on the hepatitis C virus NS3 region defined by a human monoclonal antibody. J Virol 68:4829–4836.

Moradpour D, Wakita T, Tokushige K, Carlson RI, Krawczynski K, Wands JR. 1996. Characterization of three novel monoclonal antibodies against hepatitis C virus core protein. J Med Virol 48: 234–241.

Mosely JW, Aach RD, Hollinger FB, Stevens CE, Barbosa LH, Nemo GJ, Holland PV, Bancroft WH, Zimmerman HJ, Kuo G, Choo Q-L, Houghton M. 1990. Non-A, non-B hepatitis and antibody to hepatitis C virus. JAMA 263:77–78.

Nakatsuji Y, Matsumoto A, Tanaka E, Ogata H, Kiyosawa K. 1992. Detection of chronic hepatitis C virus infection by four diagnostic systems: first-generation and second-generation recombinant immunoblot assay and nested polymerase chain reaction analysis. Hepatology 16:300–305.

Napoli J, Bishop GA, McGuiness PH, Painter DM, McCaughan GW. 1996. Progressive liver injury in chronic hepatitis C infection correlates with increased intrahepatic expression of Th1-associated cytokines. Hepatology 24:759–765.

Nasoff MS, Zebedee SL, Inchauspe G, Prince AM. 1991. Identification of an immunodominant epitope within the capsid protein of hepatitis C virus. Proc Natl Acad Sci USA 88:5462–5466.

Okamoto H, Kurai K, Okada S, Yamamoto K, Lizuka H, Tanaka T, Fukuda S, Tsuda F, Mishiro S. 1992a. Full-length sequence of a hepatitis C virus genome having poor homology to reported isolates: comparative study of four distinct genotypes. Virology 188: 331–341.

Okamoto H, Sugiyama Y, Okada S, Kurai K, Akahane Y, Sugai Y, Tanaka T, Sato K, Tsuda F, Miyakawa Y, Mayumi M. 1992b. Typing hepatitis C virus by polymerase chain reaction with type-specific primers: application to clinical surveys and tracing infectious sources. J Gen Virol 73:673–679.

Sallberg M, Zhang ZX, Chen M, Jin L, Birkett A, Peterson DL, Milich DR. 1996. Immunogenicity and antigenicity of the ATPase/helicase domain of the hepatitis C virus non-structural 3 protein. J Gen Virol 77:2721–2728.

Santolini E, Migliaccio G, Monica N. 1994. Biosynthesis and biochemical properties of the hepatitis C virus core protein. J Virol 68:3631–3641.

Siemoneit K, da Silva Cardoso M, Wolpl A, Koerner K, Subanek B. 1994. Isolation and epitope characterization of human monoclonal antibodies to hepatitis C virus core protein. Hybridoma 13:9–13.

Tomai L, Failla C, Santolini E, De Francesco R, La Monica N. 1993. NS3 is a serine protease required for processing of hepatitis C virus polyprotein. J Virol 67:4017–4026.

Weiner AJ, Geysen HM, Christopherson C, Hall JE, Mason TJ, Saracco G, Bonino F, Crawford K, Marion CD, Crawford KA, Brunetto M, Barr PJ, Miyamura T, McHutchinson J, Houghton M. 1992. Evidence for immune selection of hepatitis C virus (HCV) putative envelope glycoprotein variants: potential role in chronic HCV infection. Proc Natl Acad Sci USA 89:3468–3472.

Yang P-M, Hwang L-H, Lai M-Y, Huang W-L, Chu Y-D, Chi W-K, Chiang B-L, Kao J-H, Chen P-J, Chen D-S. 1995. Prominent proliferative response of peripheral blood mononuclear cells to a recombinant nonstructural (NS3) protein of hepatitis virus in patients with chronic hepatitis C. Clin Exp Immunol 101:272–277.